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ORIGINAL ARTICLE

Oxidative stress physiology in relation to life history traits of a free-living vertebrate: the spotted snow skink, *Niveoscincus ocellatus*

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Abstract

Recent research suggests that oxidative stress, via its links to metabolism and senescence, is a key mechanism linking life history traits such as fecundity and growth with survival; however, this has rarely been put under empirical scrutiny within free-living populations. Using a wild population of live-bearing skinks, we explored how plasma antioxidant activity (OXY), reactive oxidative metabolites (ROM), and the estimated oxidative stress index are associated with female and male life history. We found that male skinks have a significantly higher ROM and estimated oxidative stress index than female skinks, but this was not accompanied by a sex difference in mortality. Both sexes showed a non-linear association between OXY and age, indicating that the oldest and youngest individuals had the lowest OXY. Interestingly, female skinks with high OXY showed a decreased probability of survival to the following season. However, we found no significant associations between female reproductive investment (litter size or litter mass) or parturition date (i.e. metabolism) and oxidative status. Combined, our results offer mixed support for a role of oxidative stress in mediating life history traits and suggest that future studies need to explore oxidative stress during vitellogenesis in addition to using an intra-individual approach to understand the cost of reproduction and patterns of aging.

Key words: antioxidants, life history, *Niveoscincus ocellatus*, oxidative stress, reptile.

INTRODUCTION

Life history theory assumes that traits and functions compete over limited resources resulting in trade-offs (Stearns 1976). For example, if an individual invests heavily in reproduction or growth, fewer resources (such as energy) are left for somatic self-maintenance and repair mechanisms, giving rise to trade-offs between these traits and longevity. However, the costs of reproduction and growth can take several forms and trade-offs can also arise because of limitation in specific micronutrients or because of constraints arising from physiological mechanisms, such as increased oxidative stress (Monaghan *et al.* 2009; Wilkin *et al.* 2009).

Oxidative stress is caused by an overrepresentation of prooxidants, such as reactive oxygen and/or nitrogen species (ROS/RNS; referred to hereafter as RS), in relation to antioxidants (Halliwell & Gutteridge 2002). In too high concentrations, RS can result in cell degradation by damag-

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ing DNA, lipids, and proteins (i.e. cellular senescence; Harman 1956; Barja 2002). This can occur as a result of several internal processes, such as infection or normal aerobic metabolism. Consequently, increased metabolic activity typically associated with reproductive effort, growth, and physical activity may speed up the rate of senescence and thus have important implications for survival. Indeed, studies on laboratory vertebrates and humans have shown that oxidative damage increases, and antioxidant activity decreases, with age (Rodríguez-Martínez *et al.* 1999; Alonso-Alvarez *et al.* 2006; Chen *et al.* 2007). However, whether this is a cause or a consequence of aging remains unclear. Furthermore, a dietary increase of antioxidants does not generally increase longevity (Selman *et al.* 2006; Pun *et al.* 2010). Thus, evidence for the free radical theory of senescence is not unequivocal (Sanz *et al.* 2006). This is particularly the case within non-laboratory species, despite the fact that such studies have given us valuable insights into the relationship between oxidative stress and life history (Robert *et al.* 2007; Bize *et al.* 2008; Olsson *et al.* 2008, 2009; Costantini *et al.* 2009; Nussey *et al.* 2009; Robert & Bronikowski 2010).

In the present study, we explored whether oxidative stress, measured as total antioxidant capacity (OXY), reactive oxygen metabolites (ROMs), and an estimated oxidative index (OI) are associated with male and female life history traits in a free-living population of an ectotherm, namely the Tasmanian spotted snow skink, *Niveoscincus ocellatus*. Ecothermic vertebrates are particularly interesting models in which to explore the links between oxidative stress and life history. This is because, in contrast with endotherms, ectothermic vertebrates have temperature-dependent metabolism, giving them greater variation in metabolism (which, to some extent, is dependent on daily variations in access to thermal opportunities) and also the opportunity to regulate their metabolism by changing their basking behavior. Furthermore, in many ectotherms, there is a strong link between thermal opportunity (and its effects on metabolism) and evolved and/or plastic life histories in different environments (Wapstra 2000; Robert & Bronikowski 2010; Wapstra *et al.* 2010). These ecotypes can increase our understanding of the role of oxidative stress in life history biology. For example, neonates from a long-lived garter snake ecotype had a more efficient DNA repair, antioxidant defense, and mitochondria compared with the short-lived ecotype (Robert & Bronikowski 2010). Second, many ectotherms have indeterminate growth, resulting in a potential cost (trade-off) throughout life compared with endotherms. However, because there is a strong positive association between body size and reproductive investment (i.e. fecundity increases rather than decreases with female age or survival (Madsen & Shine 1992; Olsson *et al.* 2001; but see Sparkman *et al.* 2007), one needs to control for size when exploring the costs of fecundity and vice versa.

The main focus of the present study was to explore: (i) sex-specific differences in ROM and OXY during the breeding season of *N. ocellatus*; (ii) covariation between female oxidative status, reproductive investment (both current litter size and total lifetime litter size), parturition date (a thermally and metabolically dependent process) and offspring viability; (iii) whether ROM increases and OXY decreases with age, as predicted from aging theories; and (iv) whether oxidative status influences survival to the subsequent breeding season.

MATERIALS AND METHODS

Study species and capture

The spotted snow skink *N. ocellatus* is a small- to medium-sized viviparous skink (weighing 3–12 g) endemic to Tasmania, Australia. The population sampled in the present study is from a well-characterized subalpine site, namely the Central Plateau (41°51' S, 146°34' E, 1200 m above sea level), situated at the altitudinal extreme of the species distribution (Wapstra & Swain 2001; Cadby *et al.* 2010). This is a largely self-contained population occurring on a lakeside and scree rockwall surrounded by unsuitable habitat (water and dense scrub). In this population, females are gravid for 3–4 months and parturition takes place in late January/early February (Wapstra *et al.* 1999; Cadby *et al.* 2010). Depending on the year, mean population litter size varies from 3.7 to 4.3, and there is a strong positive correlation between fecundity and snout-vent length (SVL; Wapstra & Swain 2001). Individuals mature, on average, after 4 years and the maximum lifespan is 11 years for females and 12 years for males (Wapstra *et al.* 2001). All lizards evaluated in the present study were part of a larger life history study that has taken place across 10 reproductive seasons, specifically from 1999/2000 to 2009/2010 (see Wapstra *et al.* 1999, 2001; Wapstra & Swain 2001; Cadby *et al.* 2010). Thus, all individuals in the population were toe clipped to allow for unique identification prior to the present study.

We assessed oxidative stress physiology during the annual reproductive cycle in the Austral summer of 2007/2008. At the end of pregnancy, all adults (see following page) in the population were caught by mealworm "fishing", identified by their unique toe clip (assigned in previous field seasons), or, when caught for the first time, given a new toe

clip code. All female skinks were weighed (±0.01 g) and SVL was measured using a digital caliper (±1 mm). In contrast, the standard field protocol for male skinks only included a measure of SVL. New individuals were sexed by the presence or absence of hemipenes. Peripheral blood was obtained using a heparinized glass capillary from the vena angularis (in the corner of the mouth). In the field, blood samples were kept on ice and were centrifuged for 10 min at 1800 rpm within 5 h of collection. Plasma was stored separately from red blood cells and immediately frozen at -20 °C. Later the same day, samples were moved to -80 °C and stored until biochemical analysis. In total, 69 female and 51 male skinks were bled.

To obtain estimates of reproductive output, gravid females were captured in the field and then returned to a laboratory (ambient temperature 16 ± 1 ºC) at the University of Tasmania, where they were held in plastic terrariums (30 × 20 × 20 cm), each containing cover and a basking light to provide a thermal gradient for basking from 16–35 ºC. Water was available *ad libitum* and lizards were fed 3 times per week on live insects and crushed fruit. Terrariums were checked twice daily for neonates. At birth, offspring were measured (mass to ±0.1 mg; SVL to ±0.01 mm), sexed by hemipene eversion and uniquely marked for future identification. Within 4 days of birth, offspring and their mothers were released back into the natural population. Females were released close to where they were caught (i.e. the closest of 12 predetermined release sites within the site). Litters of offspring were separated and released randomly at these 12 release points to control for maternal effects and microhabitat differences (see Wapstra *et al.* 2010).

Adult and offspring survival was assessed in the subsequent spring via extensive recapture of adults and offspring subsequent to emergence from hibernation (September and October 2008) and at the end of female gestation (January 2009). Recaptures were continued over 4 weeks until only marked individuals were observed over several days of survey. We also recaptured all females the following season (2009/2010) to ascertain the accuracy of this method for assigning survival. We found only 1 female that had been incorrectly assigned, indicating an accuracy of assigning survival/mortality of 97% across the study sample as a whole. Although this was not repeated for adult males and offspring, we do not expect any sex-specific differences in recapture rates. Thus, these results suggest that our estimates of survival are unlikely to be compromised by our sampling regimen.

Oxidative stress assays (ROMs and OXY)

Antioxidant capacity and oxidative damage were measured using the OXY-adsorbent and d-ROM test kits, respectively (Diacron, Grosseto, Italy). Plasma antioxidant activity is a global measure of all antioxidants circulating in the plasma, including uric acid, Alpha-tocopherol, proteins, and bilirubin. Low OXY values indicate a lower antioxidant defense of the plasma barrier compared with high OXY values. Low OXY values may come about as a result of exposure to low concentrations of RS or they may be due to senescence and/or severe diseases, which weaken an individual's capacity to upregulate the defense system. However, measuring ROMs simultaneously in the captured skinks reveals information regarding RS exposure, enabling interpretation of the biological implications of low OXY values. "Reactive oxidative metabolites" is a collective name for the hydroperoxides (ROOH) generated by the oxidation of organic molecules such as lipids, amino acids, and nucleotides. Thus, ROM values are a general indicator of cellular oxidative stress. However, ROMs also have the potential to act as oxidants themselves, thereby amplifying damage throughout the body. This is the logical reason for also using the estimated oxidative stress index (OI) in the present study, which takes both ROM and OXY into account. In the present study, OI was calculated using the following equations:

$$OI = sv_{ROMs} - sv_{OXY}$$

and

$$sv_{var} = (v_{var} - m_{var})/ds_{var}$$

where sv_{var} represents the standardized value of the parameter (either ROM or OXY), v_{var} is the raw value of the parameter, m_{var} is the population mean of the parameter, and ds_{var} is the standard deviation of the parameter. The same formula is used for both ROM and OXY (see Vassalle 2008). Consequently, low ROM and high OXY values will generate a low OI and vice versa. In humans, a high OI has been suggested to indicate a low future resistance towards oxidative stress; however, whether this applies to wild vertebrates needs to be investigated further (Vassalle 2008).

The d-ROM and OXY-adsorbent assays were performed according to the manufacturer's instructions, with minor modification. Briefly, 15 and 5 µL plasma samples were used in assays for ROMs and OXY, respectively. The incubation temperature was set to that of the preferred body temperature of *N. ocellatus* (30 °C) and samples were

incubated for 90 or 10 min for ROM or OXY, respectively. Absorbance was measured in 1 mL cuvettes at 546 nm (spectrophotometer model DU-720; Beckman Coulter, Woerden, The Netherlands), with ROM values given in mmol H_2O_2/mL plasma and OXY values given in mmol HClO/mL plasma. For more details regarding the assays, readers are directed to Costantini and Dell'Omo (2006). Because both assays uses colorimetrics, the values were standardized for initial plasma color (residuals from ROM, OI, and OXY/plasma color regressions).

Statistical analysis

All statistical analyses were performed using JMP 8 (SAS Institute, Cary, NC, USA). Sex differences in oxidative stress measures were examined using ANOVA. Given the dissociated reproductive cycles of male and female skinks (Jones *et al.* 1997) and fundamental differences in the cost of reproduction, subsequent analyses were conducted separately for each sex. Sample sizes differ slightly between tests because not all target traits could be measured in all individuals.

In this species there is a positive correlation between chronological age and SVL ($n_{females} = 51$; $r = 0.581$; $P < 0.0001$). Thus, when the effects of size and age are tested, residuals from SVL-age regressions are used instead of the raw data for SVL. Males and females show no difference in growth rate or size over time (Wapstra *et al.* 2001). Both linear and non-linear variation in ROM and OXY in relation to age were explored (i.e. age and $(age)^2$). To avoid colinearity between age and $(age)^2$, age was mean centered by subtracting the mean age from the individual age, divided by the standard deviation.

For females, we ran 2 general linear models (GLMs) with backward elimination of factors with P values >0.25 (Quinn & Keough 2002). The first set of models used OXY, ROM, or OI as response variables, and body condition (residuals from mass–SVL regression), age, $(age)^2$, age at maturity, and offspring birth date as covariates (see Table 1; $n = 30$). Birth date was included because females with early parturition have higher metabolism because of increased basking (Wapstra 2000; Wapstra *et al.* 2010). In the second set of regression models, we tested whether the oxidative stress parameters were associated with female reproductive investment ($n = 50$). Litter size and litter mass were used as response variables and ROM, OXY, and SVL standardized for age (see above) were included as covariates. Age at maturity was not included in this analysis, which is the reason for the increased sample size here. For a subset of females ($n = 27$) we had their complete lifetime reproductive performance (litter sizes), ranging between 1 and 6 breeding seasons (mean (±SE) 2.74 ± 0.30 breeding seasons). Here, we used ROM, OXY, and OI as response variables and total lifetime reproduction standardized for size (residuals from reproduction–SVL regression) as a covariate.

As with females, the general linear models for males used OXY, ROM, or OI as response variables and included SVL (standardized for age), age, and $(age)^2$ as covariates (Table 1). However, because we did not collect data on male reproductive success, this parameter was not included in the model.

Finally, 3 logistic regressions were performed, 1 with the proportion of offspring survival (recruitment) for each female and the other 2 for male and female survival to the subsequent breeding season. In all models, adult age (or SVL), ROM, and OXY were included as covariates.

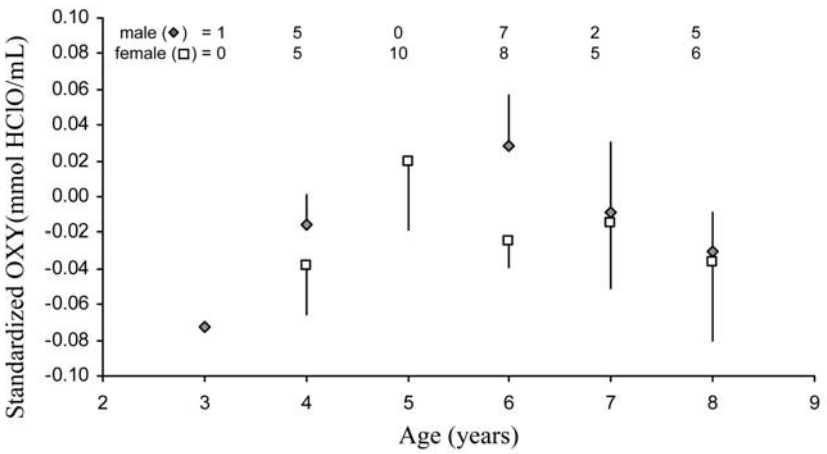

Figure 1 Age-specific antioxidant activity (OXY) in male and female snow skinks. Data show the population-level average of OXY (standardized for plasma color; see Materials and Methods) in male and female skinks with the SE indicated (–SE for females; +SE for males) and sample sizes given at the top of the graph. Details regarding the significant effects of $(age)^2$ are given in Table 1.

Table 1 Summary of the statistical models used for female and male snow skinks

Source of variation	OXY			ROM			OI		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Female skinks ($n = 30$)									
Age	1, 17	0.07	0.795	1, 30	1.88	0.179	1, 29	0.03	0.870
$(Age)^2$	1, 17	6.79	0.019	1, 30	3.04	0.091	1, 14	0.04	0.843
Age at maturity	1, 14	0.00	0.999	1, 25	0.02	0.877	1, 30	0.58	0.452
Parturition date	1, 17	1.78	0.147	1, 14	0.52	0.851	1, 17	0.91	0.538
Condition	1, 15	0.18	0.680	1, 26	0.57	0.456	1, 15	0.23	0.637
Male skinks ($n = 20$)									
Age	1, 15	0.00	0.698	1, 17	0.32	0.577	1, 16	0.01	0.935
$(Age)^2$	1, 15	4.93	0.040	1, 16	0.00	0.968	1, 16	1.08	0.314
SVL	1, 16	0.35	0.555	1, 18	0.57	0.461	1, 15	0.33	0.573

General linear models with plasma antioxidant activity (OXY), reactive oxidative metabolites (ROM), and oxidative stress index (OI) as response variables. For both females and males, there was a significant effect of $(age)^2$ on OXY. This indicates that the oldest and the youngest lizards have the highest antioxidant activity. Results are shown prior to elimination; the order of elimination is indicated by the *df* of the model (the lowest value was eliminated first and the highest *df* indicates the final model).
SVL, snout-vent length.

RESULTS

Interindividual variation and sex differences in oxidative stress status

Male and female skinks differed significantly in terms of the concentration of circulating oxidative metabolites (ROM) and OI, with males having higher ROM concentrations (ANOVA: $F_{1,116} = 7.36$, $P = 0.008$) and a higher OI (ANOVA: $F_{1,114} = 5.21$, $P = 0.024$) than females (Table 2). There was no sex difference in OXY (ANOVA: $F_{1,114} = 0.53$, $P = 0.467$; Table 2).

Both female and male skinks showed a significant association between OXY and $(age)^2$ (Table 1; Fig. 1). The significant effect of the quadratic function showed a declining pattern, indicating that skinks in their middle age had the highest antioxidant activity, whereas the youngest and oldest individuals had the lowest activity. In female skinks, parturition date, age at maturity, linear age, and body condition had no effect on OXY. Similarly, age or size (corrected for age) was not associated with OXY in male skinks (Table 1). None of the factors significantly influenced ROM or OI in either female or male skinks (Table 1).

Female reproductive investment and oxidative stress

Independent of age (residuals from SVL–age regression), larger females gave birth to larger litters ($n = 49$, $r = 0.465$, $P = 0.0008$), had larger offspring ($n = 49$, $r = 0.324$, $P = 0.023$), and had a higher total litter mass ($n = 49$, $r = 0.532$, $P < 0.0001$). Neither ROM, OXY, nor OI were associated with female litter size or litter mass (all $P > 0.170$).

In total, 168 offspring were released from 50 females and, of these offspring, 50 survived (~30%) to the subsequent summer (2008/2009). Female ROM and OXY during pregnancy did not influence offspring survival ($\chi^2 = 2.17$ and $P = 0.140$ for ROM; $\chi^2 = 0.00$ and $P = 0.957$ for OXY). Survival was greater for offspring from older females (logistic regression: $\chi^2 = 7.80$, $P = 0.005$; Fig. 2). This effect was independent of female size ($\chi^2 = 0.07$, $P = 0.793$) and average offspring size ($\chi^2 = 0.01$, $P = 0.903$).

For a subset of females ($n = 27$), the total number of offspring produced (from the first year of breeding until summer 2008; from 1 to 6 breeding seasons) was known and ranged between 1 and 24 offspring (see Materials

Table 2 Results of oxidative stress assays for male and female snow skinks

	Females ($n = 69$)	Males ($n = 50$)
ROM (mmol H_2O_2/mL plasma)	24.86 ± 0.98	27.51 ± 1.15
OXY (mmol HClO/mL plasma)	0.28 ± 0.01	0.27 ± 0.01
OI	-0.20 ± 0.18	0.27 ± 0.22

Data are the mean ± SE. ROM, reactive oxygen metabolites; OXY, antioxidant capacity; OI, oxidative stress index (estimated from the ROM and OXY as described in Materials and Methods).

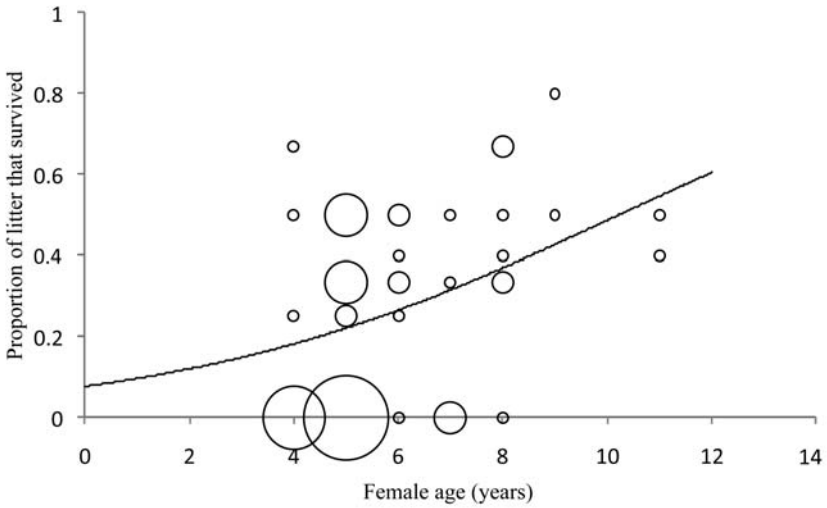

Figure 2 Older female snow skinks have higher offspring recruitment. The recruitment of offspring to the subsequent year was independent of female litter size and average offspring size. The circles represent the actual proportion of the litter recruited; the solid line shows the increasing probability of recruitment with increasing female age. The size of the circles indicates the sample size at each point (smallest circles $n = 1$; largest circles $n = 8$).

and Methods). Because body size and total lifetime reproduction were highly associated ($r = 0.79$, $P < 0.0001$), total lifetime reproduction was standardized for size. Relative total fecundity had no significant effect on ROM ($F_{1,24} = 2.99$, $P = 0.096$), OXY ($F_{1,23} = 2.41$, $P = 0.132$), or OI ($F_{1,23} = 0.47$, $P = 0.498$). The relationship between ROM and relative total fecundity was weakly negative, suggesting that those with a relatively high reproduction for their size had a lower degree of oxidative damage. If relative lifetime fecundity was standardized for the number of breeding seasons (i.e. average fecundity) rather than size, no relationship was evident ($F_{1,25} = 0.03$, $P = 0.861$ for ROM).

Survival and oxidative stress

There was no difference in survival to the following breeding season between male and female skinks (45% [23/51] and 49% [34/69] survival, respectively; $\chi^2 = 0.21$, $P = 0.650$). Females that showed a high OXY were significantly less likely to be found alive in the next year; for example, the mean (±SE) OXY concentration in surviving female skinks was 0.24 ± 0.01 mmol HClO/mL plasma, compared with 0.31 ± 0.02 mmol HClO/mL plasma in the skinks that died ($\chi^2 = 9.06$, $P = 0.003$; Fig. 3). However, no significant effect of OXY concentrations on survival was seen in male skinks (0.27 ± 0.02 vs. 0.27 ± 0.01 mmol HClO/mL

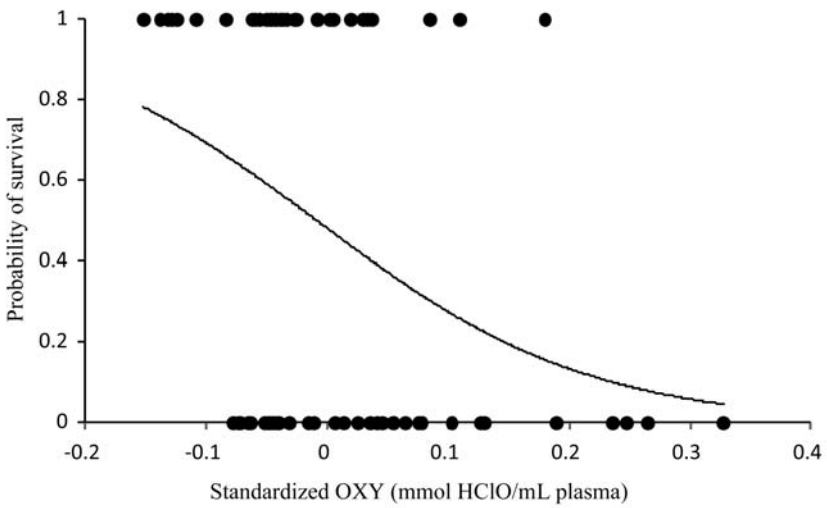

Figure 3 A negative relationship was found between the antioxidant activity (OXY) in female skinks and their survival to the following breeding season. Points represent actual survival; the solid line represents the decrease in probability of survival with increasing OXY.

plasma in surviving vs. dead skinks, respectively; $\chi^2 = 0.05$, $P = 0.821$). Survival was not significantly affected by ROM in either female ($\chi^2 = 0.79$, $P = 0.650$) or male ($\chi^2 = 0.46$, $P = 0.495$) skinks. Because of the significant effect of OXY on female survival and the lack of an effect of ROM, female skinks with a lower OI were less likely to survive ($\chi^2 = 7.24$, $P = 0.007$).

Neither body length (SVL; $\chi^2 = 2.11$, $P = 0.146$ for males; $\chi^2 = 0.32$, $P = 0.573$ for females) nor age ($\chi^2 = 0.75$, $P = 0.387$ for males; $\chi^2 = 0.63$, $P = 0.426$ for females) influenced adult survival. In contrast with theories suggesting that high reproduction increases mortality, we found no effect of litter size during 2007/2008 or the total number of offspring produced until 2007/2008 (standardized for SVL) on the probability of female survival to the subsequent 2008/2009 breeding season ($n = 50$, $\chi^2 = 0.47$, $P = 0.494$ for litter size 2007/2008; $n = 27$, $\chi^2 = 0.12$, $P = 0.726$ for standardized total litter size).

DISCUSSION

There is a strong interest in the link between variation in life history traits and oxidative stress, but data from natural populations are still scarce. The main focus of the present study was to explore whether there are any sex-specific differences in OXY, ROMs, and the OI and whether these traits are related to fecundity, age, and survival in a natural population of an ectothermic vertebrate.

Males and females show many differences in life history traits and physiology. Thus, it is not surprising that *N. ocellatus* exhibits sex differences in ROM and OI, with male skinks having significantly higher ROM concentrations and OI than female skinks. These differences may be a consequence of sex steroids' effect on RS production. Specifically, male and female sex steroids have been shown to have opposite effects on RS production, with estrogens reducing RS and testosterone increasing RS (Gupta & Thapliyal 1985; Viña *et al.* 2005; Tobler & Sandell 2009). Because snow skinks have a dissociated reproductive cycle, reproductive sex steroids peak at different times of the year (Jones *et al.* 1997). In males, testosterone is highest during spermatogenesis in mid to late summer, when we sampled the males. At the time of sampling (at the same time of year), the females were late-stage pregnant, with increased progesterone and estrogen concentrations (Jones *et al.* 1997). This could explain the increased oxidative damage in males; however, it does not explain why there was no sex difference in OXY. Such sex-specific differences at single time points in the year are supported by work on Galápagos marine iguanas, which revealed sex-specific differences in ROM and OXY, with the strength of the effects depending on when in the reproductive cycle the iguanas were sampled (Costantini *et al.* 2009). Thus, further studies that integrate oxidative measures with hormonal profiles are required to understand the causal mechanisms, seasonal differences, and the biological significance of sex differences in ROM and OI revealed in the present study.

According to the oxidative stress theory of aging, oxidative damage is often found to increase, whereas antioxidant activity decreases, with age (Beckman & Ames 1998; Finkel & Hoolbrook 2000), but a causal relationship is yet to be fully elucidated. In the present study, ROM and OI did not covary with age in either male or female skinks, and age itself was not a predictor of survival. Interestingly, in both males and females, OXY was found to have a significant, non-linear relationship with age, with the youngest and oldest individuals in the population having a significantly lower antioxidant plasma barrier compared with middle-aged individuals. That older individuals have a reduced antioxidant defense follows the prediction that the antioxidant system declines in aging individuals; however, why younger individuals have a low value is less clear. One possible explanation is that antioxidant defense increases early in life, reaches a plateau in middle age, and then decreases as a consequences of senescence. Another possibility is that the variations are the result of biased mortality (Noordwijk & de Jong 1986; Bouwhuis *et al.* 2009). Specifically, younger cohorts are likely to include low-quality individuals, whereas these individuals may have been removed from middle-aged cohorts, thus reducing the number of low-quality individuals susceptible to oxidative stress. However, the survival data do not fully support this interpretation. Instead, independent of age, female probability of survival was strongly reduced if the female had a high level of antioxidant activity the previous year. This suggests that a high antioxidant activity is an active or passive upregulation in response to increased RS caused by, for example, disease, diet change, or dehydration (e.g. Johnson 2004; Cohen *et al.* 2009; Cohen & McGraw 2009). Thus, in this species, a high OXY is not an indicator that an individual has better protection; rather, it is a marker that something is wrong and this decreases the individual's probability of surviving to the following season.

Both ectotherms and endotherms increase their metabolic rate and oxygen consumption during pregnancy (Beauchat & Vleck 1990; DeMarco & Guilette 1992; Speakman 2008; see also Munns & Daniels 2007). This is expected to cause an increase in RS leakage and thereby possibly also oxidative stress (Cadenas & Davies 2000). However, in female skinks, no significant covariations were found between

ROM, OXY, or OI and current litter size, lifetime reproductive output, or offspring survival. A potential explanation for this lack of effect is that mitochondrial uncoupling proteins alleviate RS leakage (Echtay *et al.* 2002; Schwartz *et al.* 2008). Indeed, it was recently shown that highly fecund female painted dragons (*Ctenophorus pictus*) had reduced mitochondrial superoxide production (Olsson *et al.* 2009). In the present study, skinks with relatively high reproduction for their size showed a tendency to have reduced ROM, although this relationship failed to reach statistical significance ($P = 0.096$). Despite the fact that this relationship was not significant, it further indicates that fecundity during pregnancy may not increase oxidative stress. In comparison, female alpine swifts (*Apus melba*) with a higher resistance to oxidative stress laid larger clutches (Bize *et al.* 2008). In this case, high resistance is indicated by lower hemolysis of red blood cells in response to free radical attack, which depends on both antioxidant protection and the fatty acid composition of the cell membranes (Bize *et al.* 2008). In contrast with the results of the present study regarding lizard survival and antioxidant activity, the female swifts showed no association between susceptibility to oxidative stress and survival (Bize *et al.* 2008). In another study, on free-ranging Soay sheep, no association was found in females between lipid peroxidation and reproduction, age, current parasite burden, or body mass (Nussey *et al.* 2009). Unfortunately, no links with survival were explored in that study. Other studies that have experimentally manipulated reproductive effort and measured antioxidant activity or resistance to oxidative stress have reported mixed results (Salomon *et al.* 2001; Alonso-Alvarez *et al.* 2004; Wiersma *et al.* 2004).

The results described above do not completely rule out an oxidative cost of gestation. For example, a fecundity-independent metabolic cost of pregnancy has been shown in the viviparous snake *Vipera aspis* (Ladyman *et al.* 2003). Here, metabolic rate was shown to increase with temperature in pregnant females (i.e. those that exposed themselves for longer basking times), independent of fecundity. In snow skinks, ovulation is synchronous (Jones *et al.* 1997); therefore, variation in parturition dates (i.e. gestation length) is driven by variation in female basking behavior (Wapstra 2000; Wapstra *et al.* 2010). Consequently, increased basking increases the temperature-dependent metabolic rate and also embryonic development. We therefore examined the importance of parturition date on the oxidative stress parameters but, contrary to expectation, there were no significant links. Thus, the present study does not support the suggestion that increased metabolic rate (independent of fecundity) or high fecundity (independent of basking behavior) imposes an increased oxidative stress. Further studies should explore the oxidative physiology during vitellogenesis, which is the time when the largest physiological cost of female reproduction is likely to occur (Shine 1980; Olsson *et al.* 2001).

To summarize, the present study on a natural population of a scincid lizard showed that: (i) males had significantly higher ROM concentrations and a higher OI than females, but there were no sex differences in antioxidant capacity or survival; (ii) there was no association between female reproductive investment and oxidative stress physiology during pregnancy; (iii) both male and female skinks show a significant non-linear association between age and OXY; and (iv) females with higher antioxidant activity showed lower survival probability independent of age. Finally, the present cross-sectional study provides a promising framework for future investigations into the causal links between oxidative stress, hormones, and life history.

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REFERENCES

Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B, Sorci G (2004). Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecology Letters* **7,** 363–8.

Alonso-Alvarez C, Bertrand S, Devevey G *et al.* (2006). An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution* **60,** 1913–24.

Barja G (2002). Rate of generation of oxidative stress-related damage and animal longevity. *Free Radical Biology Medicine* **33,** 1167–72.

Beckman KB, Ames BN (1998). The free radical theory of aging matures. *Physiological Reviews* **78,** 547–81.

Beuchat CA, Vleck D (1990). Metabolic consequences of viviparity in a lizard, *Sceloporus jarrovi*. *Physiological Zoology* **63,** 555–70.

Bize P, Devevey G, Monaghan P, Doligez B, Christe P (2008). Fecundity and survival in relation to resistance

to oxidative stress in a free-living birds. *Ecology* **89,** 2584–93.

Bouwhuis S, Sheldon BC, Verhulst S, Charmantier A (2009). Great tits growing old: Selective disappearance and the partitioning of senescence to stages within the breeding cycle. *Proceedings of the Royal Society of London: Series B* **276,** 2769–77.

Cadby CD, While GM, Hobday A, Uller T, Wapstra E (2010). The effect of climatic variation at the local, regional and global scale on offspring size and date of birth in a terrestrial reptile. *Integrative Zoology* **5,** 163–74.

Cadenas E, Davies KJA (2000). Mitochondrial free radical generation, oxidative stress and ageing. *Free Radical Biology Medicine* **29,** 222–30.

Chen J-H, Halesy CN, Ozanne SE (2007). DNA damage, cellular senescence and organismal ageing: Causal or correlative? *Nucleic Acids Research* **35,** 7417–28.

Cohen AA, McGraw KJ (2009). No simple measures for antioxidant status in birds: Complexity in inter- and intraspecific correlations among circulating antioxidant types. *Functional Ecology* **23,** 310–20.

Cohen AA, McGraw KJ, Robinson WD (2009). Serum antioxidant levels in wild birds very in relation to diet, season, life history strategy and species. *Oecologia* **161,** 673–83.

Costantini D, Dell'Omo G (2006). Environmental and genetic components of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *Journal of Comparative Physiology B* **176,** 575–9.

Costantini D, Dell'Omo G, de Filippis SP *et al.* (2009). Temporal and spatial covariation of gender and oxidative stress in the Galapagos land iguana *Conolophus subcristatus*. *Physiological and Biochemical Zoology* **82,** 430–7.

DeMarco V, Guillette Jr LJ (1992). Physiological cost of pregnancy in a viviparous lizard (*Sceloporous jarrovi*). *Journal of Experimental Zoology* **262,** 383–90.

Echtay KS, Roussel D, St-Pierre J *et al.* (2002). Superoxide activates mitochondrial uncoupling proteins. *Nature* **415,** 96–9.

Finkel T, Hoolbrook NJ (2000). Oxidants, oxidative stress, and the biology of ageing. *Nature* **408,** 239–47.

Gupta BBPd, Thapliyal JP (1985). Role of thyroid and testicular hormones in the oxidative metabolism of the Indian garden lizard, *Calotes versicolor*. *General and Comparative Endocrinology* **58,** 20–7.

Halliwell B, Gutteridge JMC (2002). *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford, UK.

Harman D (1956). Aging: A theory based on free radical and radiation therapy. *Journal of Gerontology* **11,** 298–300.

Johnson JD (2004). Urogenital systems. In: Girling SJ, Raiti P, eds. *BSAVA Manual of Reptiles*. British Small Animal Veterinary Association, Gloucester, UK, pp.18–39.

Jones SM, Wapstra E, Swain R (1997). Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology* **108,** 271–81.

Ladyman M, Bonnet X, Lourdais O, Bradshaw D, Naulleau G (2003). Gestation, thermoregulation, and metabolism in a viviparous snake, *Vipera aspis*: Evidence for fecundity-independent costs. *Physiological Biochemical Zoology* **76,** 497–510.

Madsen T, Shine R (1992). Determinants of reproductive success in female adders, *Vipera berus*. *Oecologia* **92,** 40–7.

Monaghan P, Metcalfe NB, Torres R (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters* **12,** 75–92.

Munns S, Daniels C (2007). Breathing with big babies: Ventilation and oxygen consumption during pregnancy in the lizard *Tiliqua rugosa*. *Physiological Biochemical Zoology* **80,** 35–45.

Noordwijk AJ, de Jong G (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *American Naturalist* **128,** 127–42.

Nussey DH, Pemberton JM, Pilkington JG, Blount JD (2009). Life history correlates of oxidative damage in a free-living mammal population. *Functional Ecology* **23,** 809–17.

Olsson M, Shine R, Wapstra E (2001). Costs of reproduction in a lizard species: A comparison of observational and experimental data. *Oikos* **93,** 121–5.

Olsson M, Wilson M, Uller T *et al.* (2008). Free radicals run in lizard families. *Biology Letters* **4,** 186–8.

Olsson M, Wilson M, Uller T, Mott B, Isaksson C (2009). Variation in levels of reactive oxygen species is explained by maternal identity, sex, and relative clutch size in a lizard. *Naturwissenschaften* **96,** 25–9.

Pun PBL, Gruber J, Tang SY *et al.* (2010). Ageing in nematodes: Do antioxidants extend lifespan in *Caenorhabditis elegans*? *Biogerontology* **11,** 17–30.

Quinn G, Keough M (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.

Robert KA, Bronikowski AM (2010). Evolution of senescence in nature: Physiological evolution in populations of garter snake with divergent life histories. *American Naturalist* **75,** 147–59.

Robert KA, Brunet-Rossini AK, Bronikowski AM (2007). Testing the free radical theory of aging hypothesis: Physiological differences in long-lived and short-lived colubrid snakes. *Aging Cell* **6,** 395–404.

Rodríguez-Martínez MA, García-Cohen EC, Briones A *et al.* (1999). Changes in plasma oxidative state with age and their influence on contractions elicited by noradrenaline in the rat tail artery. *Life Sciences* **65,** 915–24.

Salmon AB, Marx DB, Harshman LG (2001). A cost of reproduction in *Drosophila melanogaster*: Stress susceptibility. *Evolution* **55,** 1600–8.

Sanz A, Pamplona R, Barja G (2006). Is the mitochondrial free radical theory of aging intact? *Antioxidant and Redox Signalling* **8,** 582–99.

Schwartz TS, Murray S, Seebacher F (2008). Novel reptilian uncoupling proteins: Molecular evolution and gene expression during cold acclimation. *Proceedings of the Royal Society of London: Series B* **275,** 979–85.

Selman C, McLaren JS, Meyer C *et al.* (2006). Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mechanics of Ageing and Development* **127,** 897–904.

Shine R (1980). Costs of reproduction in reptiles. *Oecologia* **46,** 92–100.

Sparkman AM, Arnold SJ, Bronikowski AM (2007). An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans. Proceedings of the Royal Society of London: Series B* **271,** 943–50.

Speakman JR (2008). The physiological costs of reproduction in small mammals. *Philosophical Transaction of the Royal Society B* **363,** 375–98.

Stearns SC (1976). Life-history tactics: A review of the ideas. *Quarterly Review of Biology* **51,** 272–8.

Tobler M, Sandell MI (2009). Sex-specific effects of prenatal testosterone on nestling plasma antioxidant capacity in the zebra finch. *Journal of Experimental Biology* **212,** 89–94.

Vassalle C (2008). An easy and reliable automated method to estimate oxidative stress in the clinical setting. In: Armstrong D, ed. *Methods in Molecular Biology*, *Vol. 47: Advanced Protocols in Oxidative Stress I.* Humana Press, New York, pp. 31–9.

Viña J, Borrás C, Gambini J, Sastre J, Pallardó FV (2005). Why females live longer than males: Control of longevity by sex hormones. *Science of Aging Knowledge Environment* **23,** pe17.

Wapstra E (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology* **14,** 345–52.

Wapstra E, Swain R (2001). Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology* **35,** 194–203.

Wapstra E, Swain R, Jones SM, O'Reilly JM (1999). Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **47,** 539–50.

Wapstra E, Swain R, O'Reilly JM (2001). Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **2001,** 646–55.

Wapstra E, Uller T, While G, Olsson M, Shine R (2010). Giving offspring a head start in life: Field and experimental evidence for selection on maternal basking behaviour in lizards. *Journal of Evolutionary Biology* **23,** 651–7.

Wiersma P, Selman C, Speakman JR, Verhulst S (2004). Birds sacrifice oxidative protection for reproduction. *Proceedings of the Royal Society of London: Series B* Supplement **271**, S360–63.

Wilkin TA, Gosler AG, Garant D, Reynolds SJ, Sheldon BC (2009). Calcium effects on life-history traits in a wild population of the great tit (*Parus major*): Analysis of long-term data at several spatial scales. *Oecologia* **159,** 463–72.